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Filamentous Fungi Associated With Holothurians From the Sea of Japan, Off the Primorye Coast of Russia

MICHAEL V. PIVKIN

Pacific Institute of Bioorganic Chemistry, Far East Branch of the Russian Academy of Sciences, 690022, Vladivostok, Russia

Abstract. Holothurians (*Holothurioidea, Echinodermata*) are known to contain triterpene glycosides, which show antifungal activity. Nevertheless, fungi can be isolated from all organs of holothurians. During 1995–1996, mycelial fungi from several Far-Eastern holothurians—*Apostichopus japonicus, Eupentacta fraudatrix, Cucumaria japonica*—were collected from the Sea of Japan near the coast of Primorye (Russia) and studied. Twenty-seven species of marine fungi, mostly facultative ones belonging to the mitosporic fungi, were isolated from the holothurians and identified. Fungi isolated from the holothurian surface were more diverse and abundant than those from internal organs and coelomic fluids. Of the holothurians studied, *Cucumaria japonica* was poorest in abundance and diversity of fungi. The fungi *Cladosporium brevicompactum* and *C. sphaerospermum* were common in the holothurian coelom. Because of their high proteolytic activity, these fungi may be pathogenic to holothurians. The detritovorus holothurian *A. japonicus* was shown to modify the fungal assemblages within the marine bottom sediments.

Introduction

Interest [1]in the interactions between echinoderms and other species of marine organisms has intensified in the past few years. In addition to the trophic interrelationships of echinoderms within marine communities (Levin and Voronova, 1979) and diseases caused by bacteria, protists, and other parasites (Jangoux, 1987; Skadsheim *et al.*, 1995), benign, symbiotic interactions between echinoderms and bacteria have received much attention (Bauer and Agertr, 1994; McKenzie and Kelly, 1994; Newton and McKenzie, 1995; Kelly and McKenzie, 1995; Kelly *et al.*, 1995; Thorsen, 1995). However, there is very little information about the fungi of echinoderms. Mortensen (1909) described a peculiar disease in the Antarctic cidaroid echinoids, genera *Rhynochocydaris* and *Ctenocidaris*, which was caused by the fungus-like organism *Echinophyces mirabilis*. More recently, fungi were found to damage the spines of the sea urchins *Diadema antillarum* (Mortensen, 1940) and *Strongylocentrotus franciscanus* (Johnson and Charman, 1970). There is some evidence that fungi are present in the intestine of a sea cucumber, *Apostichopus japonicus*, from the Sea of Japan, but no data are available on their abundance or taxonomy (Levin, 1982).

Despite the lack of data, the presence of fungi in holothurians is of potential importance. Holothurians are known to contain triterpene glycosides, which show fungitoxic, hemotoxic, and cytotoxic activities (Stonik and Elyakov, 1988). The diverse biological activities of the triterpene glycosides depend upon their specific binding to Δ^{-5} sterols (Kalinovskaya *et al.*, 1983; Kalinin *et al.*, 1994, 1996). Such sterols are common in most fungi, but holothurian fungi must be assumed to contain some other sterols that will not bind to holothurian glycosides. In addition, fungi adapted to the effects of triterpene glycosides might produce secondary metabolites having a similar type of action. Thus, the isolation, in pure culture, of fungal strains from holothurians, and a definition of their species diversity would give us an insight into the biochemical mechanisms by which the fungi adapt to triterpene glycosides.

This study had three aims. The first was to establish that fungi are found in and on holothurians—a surprising phenomenon since holothurians are well known as producers of fungitoxic glycosides. The second aim was to prepare a list

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E-mail: Elyakov@stl.ru

of fungi isolated from the holothurians collected along the coast of the Primorye territory in the Sea of Japan. Such a list is basic to any mycological research. In mycology, the substrate from which a fungus was isolated, as well as its area of distribution, are characteristics used in identification, as are the morphological features and genetic distinctions of any species. The final goal was to be able to speculate rationally about the role of holothurian fungi in the environment.

Materials and Methods

Sampling

We studied the fungi from three species of Far-Eastern holothurians: *Apostichopus japonicus*, *Eupentacta fraudatrix*, and *Cucumaria japonica*. In 1995–1996, 10 animals of each species were collected at each of three sites along the Russian coast of the Sea of Japan: (1) the Gulf of Opritchnik, near Cape Skalistyi (44°26′3″N, 135°59′5″E; depth 12–14 m); (2) the Primorye coast (45°51′1″N, 136°37′3″E; depth 111 m); (3) in Trinity Bay of Peter the Great Bay (42°34′6″N, 130°57′58″E; depth 1.5–15 m). Specimens were placed in sterile polyethylene bags and either processed immediately or stored in a refrigerator at 0°–3°C for not more than 48 h.

Two additional types of material were collected at site 3. Feces from *A. japonicus* were obtained by placing five animals in sterile polyethylene cages suspended in the sea at the site for 6 h, then collecting the fecal material in sterile polyethylene bags. Bottom sediments from the habitat of this species were also collected in sterile bags.

Isolation of fungi

Fungi were plated and cultured from five types of material: from three holothurian tissues—surface muscle, intestine, and coelomic fluid—and from feces and bottom sediment.

Holothurian specimens were washed five times in sterile seawater, and 0.5-cm^3 samples of surface muscle and internal organs were dissected. Pieces of these tissues were placed on HA medium (Table 1) in sterile petri dishes. Coelomic fluid was extracted with a sterile syringe and inoculated into HA medium. Fungi from bottom sediments, fecal material, and stomach contents were isolated by dilution plating (Steele, 1967). Colonies from all of these cultures were then reseeded into tubes of Tubaki's medium (Table 1).

Fungal identification and diversity calculation

The fungi were re subcultured and identified to species using slide cultures as well as morphological characteristics of the colony. Use of slide cultures allows microscopic examination of intact fungal reproductive structures. In this method, melted MA medium (Table 1) is placed on a sterile microscope slide at sufficiently high temperature, and in sufficient amount, to cover half the length of the slide surface and three quarters of its width with a thin, flat layer of agar. When the agar solidified, the fungus was inoculated on the surface by streaking spores in two parallel rows extending the length of the agar layer. The slide cultures were incubated at 22°C in a humid chamber and allowed to grow until reproductive structures appeared (3–7 days). Each fungal isolate was identified using common keys (Thom and Raper, 1945; Raper and Thom, 1949; Ellis, 1971; Gams, 1971; De Hoog, 1972; Samson, 1974; Shmotina and Golovleva, 1974; Tulloch, 1976; Kohlmeyer and Kohlmeyer, 1979; Ramirez, 1982; Bilay and Koval, 1988; Egorova, 1988), and the following media: Czapek's (Ramirez, 1982), MPCA, Tubaki's and MA (Table 1). The equitability and diversity of fungal species from each holothurian species were calculated using the Shannon-Weiner diversity index (Magurran, 1988).

Proteolytic activity of holothurian fungi

Production of extracellular proteases was determined by growing three cultures of each isolate at 22°C on GM media (Table 1) in tubes of 18 mm diameter (Bilay, 1982). Liquefying zones were measured in 7 and 14 days.

Table 1

Media used in the culture of holothurian fungi

Abbreviation	Medium
HA	*Agar with holothurian broth:* 100 g of a holothurian sample boiled for 30 min in a liter of seawater, filtered, brought up to a liter of distilled water; pH adjusted to 6.8 with NaOH, 20 g of agar added; sterilized at 121°C for 20 min; half a million units of penicillin and 0.5 g of streptomycin added after sterilization.
MPCA	*Potatoes-carrot agar with seawater and yeast extract:* 20 g of potatoes and 20 g of carrot boiled for 30 min in a liter of seawater, crushed, brought up to a liter of distilled water; 0.5 g of yeast extract and 20 g of agar added; pH adjusted to 7.5 with NaOH, sterilized at 121°C for 20 min.
GM	*Gelatinous medium:* 2 g of gelatin in 100 ml of seawater; pH adjusted to 6.8 with NaOH; sterilized at 121°C for 20 min (Bilay, 1982).
MA	*Malt seawater agar:* 5% malt extract agar made up with seawater; pH adjusted to 7.5 with NaOH; sterilized at 112°C for 30 min (Hyde *et al.*, 1987).
	Tubaki's medium with natural seawater: 30 g of glucose, 1 g of peptone, 0.5 g of yeast extract, 1 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 1 liter of seawater; pH adjusted to 7.5 with NaOH; 15 g of agar added; sterilized at 112°C for 30 min (Tubaki, 1969).

Resistance of fungi to glycosides

Resistance of fungi to the action of glycosides extracted from the holothurian *Eupentacta fraudatrix* was detected by the agar diffusion method. The following fungi were tested: *Cladosporium sphaerospermum* from coelomic fluid of *E. fraudatrix*, *Aspergillus eburneocremeus* from the surface of the holothurian, *Cladosporium cladosporioides* from bottom sediments, *Aspergillus flavipes* from the alga *Fucus evanescens*, and *Candida albicans* KMM 245. Solutions of the glycoside fraction at concentrations of 0.625, 1.25, 2.5, 5, 10, 20, and 40 mg/ml were used in the tests. A total fraction of glycoside, common to these animals (Kalinin *et al.*, 1994) was kindly provided by Dr. Avilov of the Pacific Institute of Bioorganic Chemistry.

Statistical analysis

The statistical significance of the proteolytic activity of the holothurian fungi and their resistance to holothurian glycosides was analyzed by the Fisher exact probability test (Sokal and Rolf, 1981). The results of the investigation of fungal species diversity were evaluated statistically using Student's *t* test (Magurran, 1988).

Results

Species diversity of fungi from holothurians

Twenty-seven species of marine fungi were isolated from various organs and tissues of holothurians and identified (Table 2). Most of these isolates were mitosporic fungi, predominantly *Hyphomycetes* (24 spp.) and *Coelomycetes* (3 spp.). The obligate marine fungi (definition according to Kohlmeyer and Kohlmeyer, 1979) included three species: *Botryophialophora* sp. (*Hyphomycetes*), *Phialophorophoma* sp., and *Coniothirium obiones* (*Coelomycetes*). The facultative marine fungi of holothurians were represented by 24 species of "terrestrial" fungi. All of these species belong to 13 genera of the class *Hyphomycetes*.

Among the holothurians investigated, the mycota of *Eupentacta fraudatrix* were the most diverse. *Cladosporium oxysporum* and *Metarchizium anisopliae* were the fungi that occurred most frequently on the surface of these animals. *Cladosporium sphaerospermum* was the dominant species inside of *E. fraudatrix* and on the surface of *Apostichopus japonicus* and *Cucumaria japonica*. *Cladosporium brevicompactum* dominated inside of *A. japonicus* and *C. japonica* (Table 2).

Three species of fungi, *Pacilomyces puntonii*, *C. sphaerospermum*, and *Beauveria alba*, occurred on the gonads of *A. japonicus* and *E. fraudatrix*.

Proteolytic activity of some fungal strains from holothurians

The proteolytic activities of some holothurian fungal strains are listed in Table 3. Of the eight fungal species taken from the surface of the holothurian *Eupentacta fraudatrix*, only one, *Penicillium commune*, had no proteolytic activity. However, the congeneric species *P. herquei* had the highest rate of gelatinous medium liquefaction when compared to other fungal species found on the surface (although the rates for *Acremonium striatisporum* and *Alternaria alternata* were also relatively high). Fungi of the species *Cladosporium sphaerospermum* and *C. brevicompactum* isolated from internal organs of these three holothurians demonstrated higher gelatinolytic activity than the same fungal species isolated from the surface. Indeed, the activity demonstrated by *C. brevicompactum* taken from the internal organs of *Cucumaria japonica* was the highest measured in these experiments.

Resistance of fungi to glycosides of the holothurian Eupentacta fraudatrix

The inhibition of fungal growth by different concentrations of a glycoside fraction taken from *E. fraudatrix* is plotted in Figure 1. These glycosides inhibited *Candida albicans* and fungal strains from bottom sediments and from *Fucus evanescens* (curves Cal, Ccl, Afl) at the lowest concentration (0.625 mg/ml); but the growth of the fungus *Aspergillus eburneocremeus*, isolated from the surface of this holothurian, was stopped only at glycoside concentrations 5 mg/ml or higher (curve Aeb). *Cladosporium sphaerospermum*, isolated from internal organs (curve Csp), was resistant to these holothurian glycosides at 40 mg/ml, a concentration many times higher than it could be in this holothurian (Kalinin *et al.*, 1994, 1996). That fungi from various marine habitats differ in their resistance to the glycosides of *E. fraudatrix* is illustrated in Figure 2. The fungi from this holothurian (top dishes) are more resistant to its glycosides than are fungi from bottom sediments and from *Fucus evanescens* (bottom dishes).

Fungi in the intestine, feces, and habitat of the holothurian Apostichopus japonicus

The abundance and species composition of fungal propagules (conidia, spores, and pieces of mycelia) in the intestine of *A. japonicus*, in its feces, and in the marine sediments it inhabits are set out in Table 4. The abundance of fungi in the bottom sediments was ten times higher than in the intestine of this sea cucumber. However, the abundance of fungi in feces was twice that in samples of bottom sediment taken from the habitat of these holothurians. The species composition of fungi is also different in the sediment, in-

Table 2

Fungi from holothurians, species diversity and distribution

Taxon		*Eupentacta fraudatrix* S	I	C	*Apostichopus japonicus* S	I	C	*Cucumaria japonica* S	I	C
Cladosporium sphaerospermum Penz.			■	■	●	+	+	●		
C. brevicompactum Pidopl. et Deniak		+			X	●	■	■	■	■
C. atrospermum Pidopl. et Deniak		+	+		+	+	+	●		**3**
C. oxysporum Berk. et M. A. Curtis		X			+					
Alternaria alternata (Fr.) Keissl.		+			X					
Aspergillus versicolor (Vuill.) Tirab.			+		+	+	+			
A. eburneocremeus Sappa		+			+					
Epicoccum st. *Phoma* sp.		+					+			
Ulocladium sp.		+			+		**9**			
Aspergillus flavus Link							+			
Acremonium charticola (Lindau) W. Gams		+								
A. fusidioides (Nicot) W. Gams		+								
A. striatisporum (Onions & G. L. Barron) W. Gams		+								
Beauveria alba (Limber) Saccas		+	■							
Botryophialophora sp.*		+								
Coniothirium obiones Jaap*		+								
Metarchizium anisopliae (Metschn.) Sorokin var. *anisopliae* J. R. Johnst.		X								
Oidiodendron sp.		+								
Paecilomices puntonii (Vuill.) Nann.						●	**2**			
Penicillium commune Thom		+								
P. herquei Bainier et Sartory		+								
P. implicatum Biourge		+								
P. roqueforti Thom		+								
P. skrjabinii Schmotina et Golovleva		+								
Phialophorophoma sp.*		+								
Tilachlidium sp.		+								
Verticillium tenerum Ness		+		**25**						
Total number	**27**	24	4	1	8	5	6	3	1	1
†Shannon-Weiner diversity index		2.95			1.67			0.82		
Equitability		0.93			0.76			0.74		

Letters (S, I, C) indicate the holothurian site from which the fungi were isolated: S, surface; I, internal organs; C, coelomic fluid. Symbols indicate the frequency of fungal species occurrence: +, 0%–10%; X, 10%–20%; ●, 20%–40%; ■, more than 40%. Boldface numbers give summary information about the distribution of the fungi among the holothurians. For example, of the 27 fungal species identified, 25 were found on *Eupentacta fraudatrix*; 2 of the 11 species found on *Apostichopus japonicus* were exclusive to that species, and 9 were also found on *E. fraudatrix*; and 3 species of fungi were found on *Cucumaria japonica*, none exclusively.

* Obligate species of marine fungi.

† Differences in species diversity of fungi on the holothurian species are statistically significant at the 99.8% level.

testine, and feces. The intestine has the fewest species, but all of them occur in the sediment and in the feces.

Discussion

The vast majority of fungi isolated from the holothurians were facultative marine species. Only three species of obligate marine fungi were found in the holothurians, and all of them were isolated from the surface of *Eupentacta fraudatrix* (Table 2). Thus, among the holothurians investigated, the facultative marine fungi are more diverse than the obligate ones, although holothurians are rather substrate-specific. In the sea there are not many substrates suitable for fungi, and just for this reason associations between fungi and other organisms are unusual. We should therefore reconsider the general notion that the role of facultative marine fungi in ecology is of little importance.

The presence of fungi on holothurians is unexpected, because holothurian glycosides are highly fungicidal. The glycosides from *E. fraudatrix* have a higher fungicide activity than those from any other holothurian species studied (Kalinin *et al.*, 1994). We therefore determined the resistance of two fungal species from *E. fraudatrix* and two

Table 3

Proteolytic activity of fungal strains from holothurians

Source	Taxon	Proteolytic activity (cm)*
Eupentacta fraudatrix	*Acremonium striatisporum*	4.0 ± 0.2
Surface	*Penicillium commune*	0
	P. herquei	6.0 ± 0.1
	Alternaria alternata	4.5 ± 0.3
	Phialophorophoma sp.	0.8 ± 0.1
	Verticillium tenerum	1.0 ± 0.2
	Cladosporium sphaerospermum	1.0 ± 0.2
	C. brevicompactum	3.0 ± 0.1
Internal organs	*C. sphaerospermum*	6.0 ± 0.3
Apostichopus japonicus	*C. sphaerospermum*	0.5 ± 0.1
Surface	*C. brevicompactum*	0.8 ± 0.1
Internal organs	*C. sphaerospermum*	5.0 ± 0.3
	C. brevicompactum	6.0 ± 0.3
Cucumaria japonica	*C. sphaerospermum*	1.0 ± 0.2
Surface	*C. brevicompactum*	1.2 ± 0.1
Internal organs	*C. brevicompactum*	8.0 ± 0.3

* Differences in proteolytic activity of fungal strains are statistically significant at the 99% confidence level. $P < 0.001$. Values are expressed as the mean, plus or minus the standard deviation.

fungal species from other marine habitats to a glycoside fraction from *E. fraudatrix*. *Candida albicans* KMM 245 was used as a control. Fungi from *E. fraudatrix* appeared to

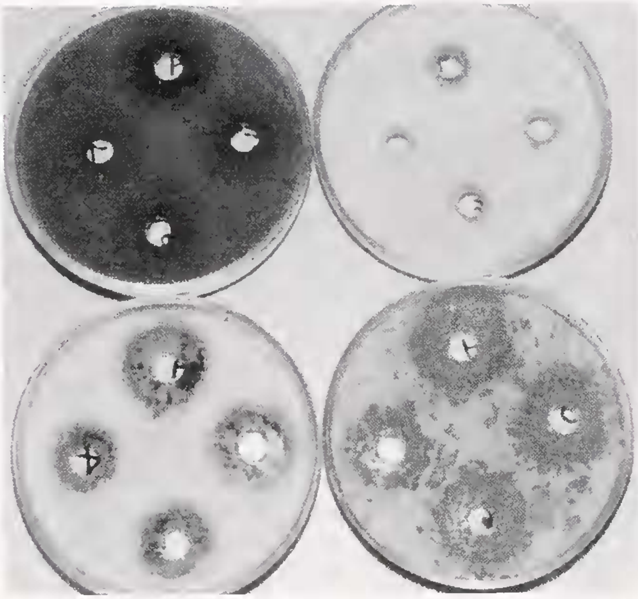

Figure 2. Inhibition of fungal growth by four concentrations of glycoside from the holothurian *Eupentacta fraudatrix*. Glycoside concentrations in each plate are (clockwise from top) 40, 20, 10, and 5 mg/ml. Top left plate: *Cladosporium sphaerospermum* isolated from coelomic fluid of the holothurian (see curve Csp in Fig. 1); Top right: *Aspergillus eburneocremeus* isolated from the surface of the holothurian (curve Aeb in Fig. 1); Bottom left: *Cladosporium cladosporioides* isolated from bottom sediments (curve Ccl in Fig. 1); Bottom right: *Aspergillus flavipes* from the alga *Fucus evanescens* (curve Afl in Fig. 1).

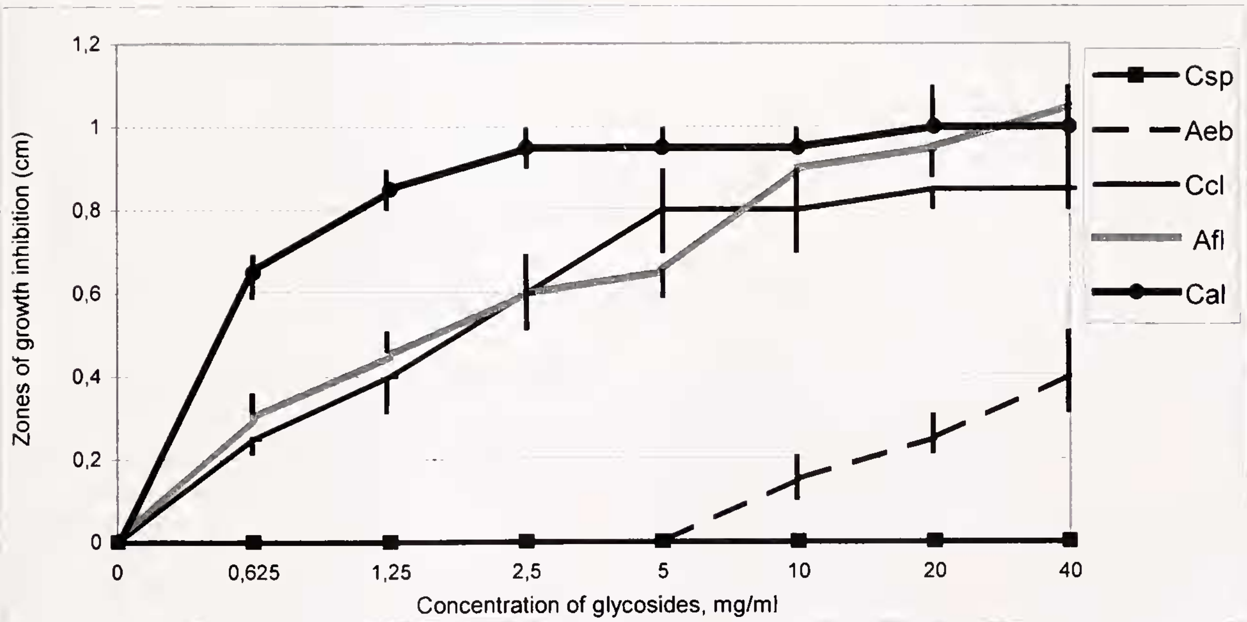

Figure 1. Inhibition of fungal growth by glycosides of the holothurian *Eupentacta fraudatrix*. Curves: Csp, *Cladosporium sphaerospermum* isolated from coelomic fluid of the holothurian. Aeb, *Aspergillus eburneocremeus* isolated from the surface of the holothurian. Ccl, *Cladosporium cladosporioides* isolated from bottom sediments. Afl, *Aspergillus flavipes* isolated from the alga *Fucus evanescens*. Cal, *Candida albicans* KMM 245. Differences in resistance of fungal strains to glycosides are statistically significant at the 99% confidence level. $P < 0.001$.

Table 4

Abundance and fungal species composition of the internal contents and feces of Apostichopus japonicus, *as well as the marine sediments it inhabits*

	Materials Examined*		
Fungal taxon	Bottom sediments (1.3×10^4)	Intestine (0.8×10^3)	Feces (2.6×10^4)
Acremonium fusidioides (Nicot) W. Gams	+	+	+
A. kiliense Grutz			+
A. trachycaulon W. Gams			+
Alternaria alternata (Fr.) Keissl.	+	+	+
Arthrynium sp.	+		+
Aspergillus flavus Link	+		
A. halophilicus C. M. Chr., Paravizas et C. R. Bej.	+		
A. niger Tiegh.	+		
A. sydowi (Bainier et Sartory) Thom et Church	+		
Chaetomium olivaceum Cooke et Ellis	+	+	+
Cladosporium atrospermum Pidopl. et Deniak	+	+	+
C. brevicompactum Pidopl. et Deniak	+	+	+
C. sphaerospermum Penz.	+	+	+
Dendryphiella arenaria Nicot	+	+	+
Oidiodendron truncatum G. L. Barron	+	+	+
Penicillium brevicompactum Dierckx	+		
P. chrysogenum Thom	+		
P. commune Thom	+	+	+
P. herquei Thom	+	+	+
P. thomii Maire	+		
Stilbella aciculosa (Ellis et Everh.) Seifert	+		+
Trichoderma aureoviride Rifai	+	+	+
T. harzianum Rifai	+	+	+
T. viride Rifai	+	+	+
Wallemia sebi (Fr.) Arx	+		
Number of species	23	13	17

* Numbers in parentheses are the number of fungal propagules per gram of material.

have more tolerance for these glycosides than did fungi from other marine substrates (Figs. 1, 2). This suggests that fungicidal glycosides may not limit the ability of certain species of fungi to grow on a holothurian.

More species of fungi inhabit the surface of holothurians than the internal organs and coelomic fluid (Table 2). The mycota of holothurians typically comprises unspecific cosmopolitan species that occur in soils and on various protein-rich substrates (Table 5), whereas the internal fungal assemblages of holothurians are rather specific. Thus, three of seven fungal species isolated from internal organs and coelomic fluid were found in only one holothurian species (Table 2). Four other species were characteristic of the internal mycota of two holothurian species. But none of these fungal species occurred in all three species of these holothurians.

Pacilomyces puntonii and *Beauveria alba* are rare species, found on human skin (De Hoog, 1972; Samson, 1974); they have been isolated from the gonads of *A. japonicus* and *E. fraudatrix*, respectively. The cosmopolitan species *Cladosporium sphaerospermum*, which occurs on various substrates, including ones of animal origin (Ellis, 1971), was also isolated from the gonads of *E. fraudatrix*. Even though the amount of glycosides in gonads can be ten times more than in other organs of these holothurians (Levin, 1982), these species of fungi were found even in such adverse conditions.

The fungal species from *E. fraudatrix* are more diverse than those from any other holothurian species studied; and as indicated above, glycosides from this holothurian have the highest fungicide activity. Triterpene glycosides from *C. japonica* are active against *Candida albicans* and *C. tropicalis* at concentrations higher than 30–50 mg/ml, but they have lower fungicidal activity than other holothurian glycosides (Kalinin *et al.*, 1994). We might expect that the toxicity of secondary holothurian metabolites would influence the species diversity of the fungi associated with these animals; but in fact, the diversity of fungi increases with growing glycoside toxicity.

The investigated holothurians differ in food specialization. *A. japonicus* is an animal that feeds on organic material that has settled to the marine floor. *E. fraudatrix* and *C. japonica* feed on organic particles suspended in the seston. Because the number and diversity of fungi in the bottom sediments are about 100 times higher than those in the water column (Steele, 1967), *A. japonicus* has contact with a much more diverse fungal assemblage than the two other holothurians encounter. Nevertheless, this condition does not provide the greater diversity of fungi associated with *A. japonicus*.

Depth of habitat might also affect fungal diversity. The most deep-sea species, *Cucumaria japonica*, has the fewest fungal species. *A. japonicus* is common at depths down to 100 m and has more diverse mycota than *C. japonica*. The greatest diversity and equitability of fungal species are characteristic of *E. fraudatrix*, which has an optimal depth of 1.5 m but can occur down to 40 m. Therefore, fungal diversity appears to decrease with the depth of the holothurian habitat.

The regions of isolation of these holothurians are also important. In our experience, the species composition of fungi isolated from *C. japonica* collected from areas around the Kuril Islands in 1998 was different from that isolated from *C. japonica* collected in Primorye waters. Fungi that

Table 5

Fungi isolated from holothurians: distribution, habitats, substrates

Taxon	Substrates and distribution	References
Acremonium charticola	Cellulose substrates, human skin (-*Cephalosporium ballagii*), widespread, unknown in the sea.	Gams, 1971
A. fusidioides	Soil, widespread, unknown in the sea.	Gams, 1971
A. striatisporum	Soil, widespread, unknown in the sea.	Gams, 1971
Alternaria alternata	On various marine substrates, widespread.	Johnson and Sparrow, 1961; Jones, 1962, 1963, 1968, 1972
Aspergillus eburneocremeus	Soil, Southeastern Asia, North and South America.	Artemtchuk, 1981; Steele, 1967; Bilay and Koval, 1988
A. flavus	Soil, humans, and animals, seawater, bottom sediments, and coastal sands, widespread.	Thom and Raper, 1945; Bilay and Koval, 1988; Steele, 1967
A. versicolor	Soil, (-*A. versicolor* var. *glauca*) pathogenic for man and animals, bottom sediments, seawater, widespread.	Bilay and Koval, 1988; Blochwitz, 1934; Thom and Raper, 1945; Steele, 1967
Beauveria alba	Insects, human skin, widespread, unknown in the sea.	De Hoog, 1972
Botryophialophora sp.	Allied to *Botryophialophora marina* that inhabits wood, test panels, and coastal sands in the Atlantic and Pacific Oceans.	Linder, 1944; Steele, 1967; Wilson, 1951
Cladosporium atrospermum	Soil, cosmopolitan on various substrates, widespread, unknown in the sea.	Ellis, 1971
C. brevicompactum	Soil, cosmopolitan on various substrates, widespread, unknown in the sea.	Ellis, 1971
C. oxysporum	Soil, cosmopolitan on various cellulose substrates, widespread, unknown in the sea.	Ellis, 1971
C. sphaerospermum	Soil species, cosmopolitan on various substrates including ones of animal origin, widespread, unknown in the sea.	Ellis, 1971
Coniothirium obiones	Saprotrophic species, Atlantic and Indian Oceans.	Kohlmeyer and Kohlmeyer, 1979
Metarchizium anisopliae var. *anisopliae*	Insects, widespread, unknown in the sea.	Latch, 1965; Tulloch, 1976
Oidiodendron sp.	Soil, plants, and others.	Ellis, 1971
Penicillium commune	Soil, contaminants from cheese and other foodstuffs, bottom sediments, seawater, widespread.	Ramirez, 1982; Raper and Thom, 1949; Steele, 1967
P. herquei	Soil, widespread, but not abundant, unknown in the sea.	Raper and Thom, 1949
P. implicatum	Soil, widespread, but not abundant, unknown in the sea.	Raper and Thom, 1949
P. roqueforti	Soil, contaminants from cheese and other foodstuffs, widespread, unknown in the sea.	Ramirez, 1982; Raper and Thom, 1949
P. skrjabinii	Soil, Far East, vicinities of Blagoveshchensk, unknown in the sea.	Shmotina and Golovleva, 1974
Pacilomices puntonii	Human skin, widespread, but not abundant, unknown in the sea.	Samson, 1974
Phialophorophoma sp.	Allied to *Phialophorophoma litoralis* that is found on cellulose substrates, and on the dead alga *Avicennia marina* var. *resinifera*, the Atlantic and Pacific Oceans	Kohlmeyer and Kohlmeyer, 1979
Tilachlidium sp.	Soil, plants, and others.	Ellis, 1971
Ulocladium sp.	Soil, plants, and others, unknown in the sea.	Ellis, 1971
Verticillium tenerum	Soil, widespread, unknown in the sea.	Gams, 1971

were prevalent in isolates from Primorye holothurians were very rare in the cucumbers from the Kuril Islands, where they occurred only in the southern collection sites.

Proteolytic activity is frequently suggested as a factor in fungal pathogenicity (St. Leger *et al.*, 1988; Monod *et al.*, 1995). The body of any holothurian contains 1.9%–9.0% of protein, mainly collagen, and the content of protein exceeds that of all other components of the holothurian body, excluding water (Levin, 1982). Therefore, the ability of fungi to damage holothurian tissues can be estimated from their proteolytic activity, thus precluding the need to inoculate the animals. In particular, the gelatinolytic activity of holothurian fungi, determined simply by measuring the liquefaction of gelatinous medium (denatured collagen), allows us to estimate their pathogenicity. The strains of *Cladosporium sphaerospermum* and *C. brevicompactum* isolated from the internal organs of these holothurians are twice as active in liquefying gelatin as are isolates from their surfaces (Table 3). Note that the fungi of these species often occur in internal organs and coelomic fluid of holothurians; in several cases, the animals contained a monoculture of these fungi species. Recall, furthermore, that *C. sphaerospermum* is resistant to glycosides of *E. fraudatrix* (Fig. 1). These facts indicate that *C. sphaerospermum* and *C. brevicompactum* may be pathogenic for holothurians.

However, the terms "pathogen" for these fungi and "host" for these holothurians must be used with care, because they imply a parasitic relationship between organisms—in this case between a macroorganism and a pathogenic microorganism. To confirm the pathogenicity of a microorganism, the classical method of Koch should be used, and this includes three steps: (1) a search for symptoms of a disease; (2) isolation of potential pathogens into pure culture; (3) artificial inoculation of this organism. In the end, a microorganism can be recognized as pathogenic if all the symptoms of a recognizable disease produced by infection completely coincide with those that appear after artificial inoculation. Such an analysis was not possible in the present study. Therefore, an analysis of gelatinolytic activity was taken as indirect evidence for the ability of a fungus to damage holothurian tissues, and on that basis, fungi isolated from coelomic fluid and internal organs were considered to be *potentially pathogenic*.

Similarly, the term "symbiont" can not be used, because no symbiosis between fungi and holothurians has yet been proven. Since, in our current state of knowledge, the relationship between fungi and holothurians cannot be described as either parasitic or symbiotic, we should consider fungi isolated from a holothurian surface to be *epiphytic*.

We may also ask whether holothurians affect the mycota of bottom sediments; in particular, can they modify the abundance and species composition of these fungi? As mentioned above, *A. japonicus* feeds on marine deposits (Levin, 1982), which include propagules of fungi. A comparison of fungi from bottom sediments and from holothurian feces revealed that *A. japonicus*, armed with the triterpene glycosides, increased the abundance of dematiaceous species of *Hyphomycetes* and eliminated some species of *Moniliaceae* from the bottom sediments (Table 3). Dematiaceous *Hyphomycetes* of the genera *Alternaria*, *Arthrynium*, and *Dendryphiella* passed through the alimentary tract of *A. japonicus* practically without loss of their viability; and the number of fungi of the genus *Cladosporium* even increased under these conditions. But not all dematiaceous *Hyphomycetes* are unaffected by transit through a holothurian. For example, *Wallemia sebi* was isolated from bottom sediments, but occurred in neither the intestine nor the feces of the sea cucumber. *Hyphomycetes* of the family *Moniliaceae* were affected more selectively: *e.g.*, fungi of the genera *Acremonium* and *Trichoderma* were resistant to passage through the sea cucumbers, whereas fungi of the genus *Aspergillus* were not found in feces of these animals. Of all the fungi of the genus *Penicillium* found in bottom sediments, only *P. herquei* and *P. commune* did not lose viability in the intestine of these sea cucumbers.

Fungi from holothurians have never been investigated before. This pioneer report describes the species compositions of fungi from holothurians and their possible ecological role.

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